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(54) **PHARMACEUTICAL COMPOSITION FOR PREVENTING AND TREATING RESTENOSIS COMPRISING PEROXIREDOXIN 2 PROTEIN AS AN ACTIVE INGREDIENT AND COMPOSITION FOR SCREENING THERAPEUTICS OF RESTENOSIS COMPRISING PEROXIREDOXIN 2 GENE OR ITS PROTEIN PRODUCT, AND METHOD FOR SCREENING THERAPEUTICS OF RESTENOSIS USING SAID COMPOSITION**

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(57) **ABSTRACT**

Disclosed herein is a pharmaceutical composition for the prophylaxis and treatment of restenosis, comprising a peroxiredoxin 2 (Prx II) protein as an active ingredient. Optionally, the composition may contain other active ingredients suppressive of restenosis at the carotid artery, the coronary artery, the peripheral artery, and the renal artery. Also, a composition and a method are provided for screening therapeutics for restenosis. This screening composition comprises a peroxiredoxin 2 gene or protein. Together with the method, the composition is useful for searching and developing therapeutics for restenosis.

FIG. 1

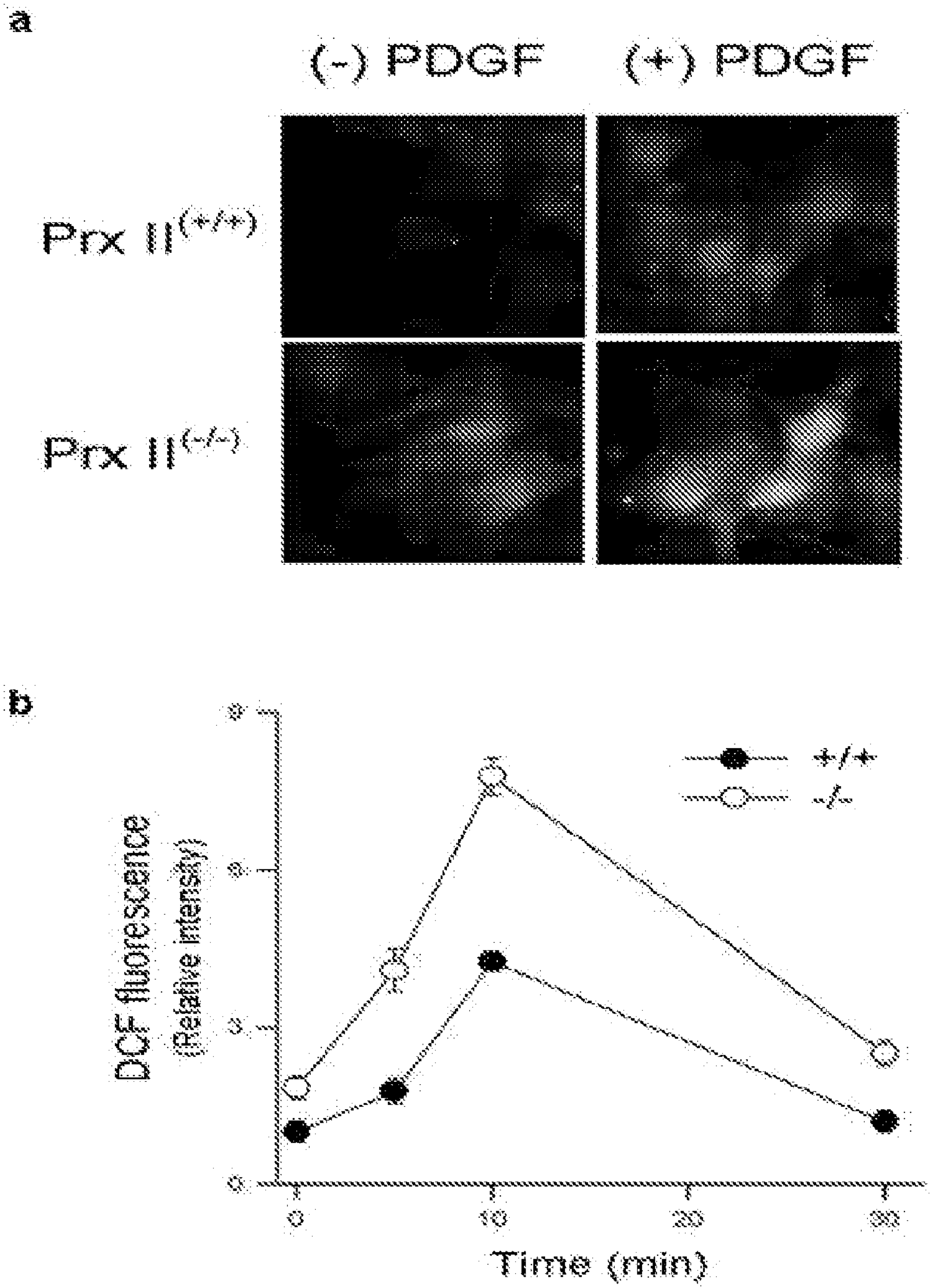

a
(-) PDGF
(+) PDGF
Prx II(+/+)
Prx II(-/-)
b
+/+
-/-
DCF fluorescence
(Relative intensity)
0
10
20
30
Time (min)

FIG. 2

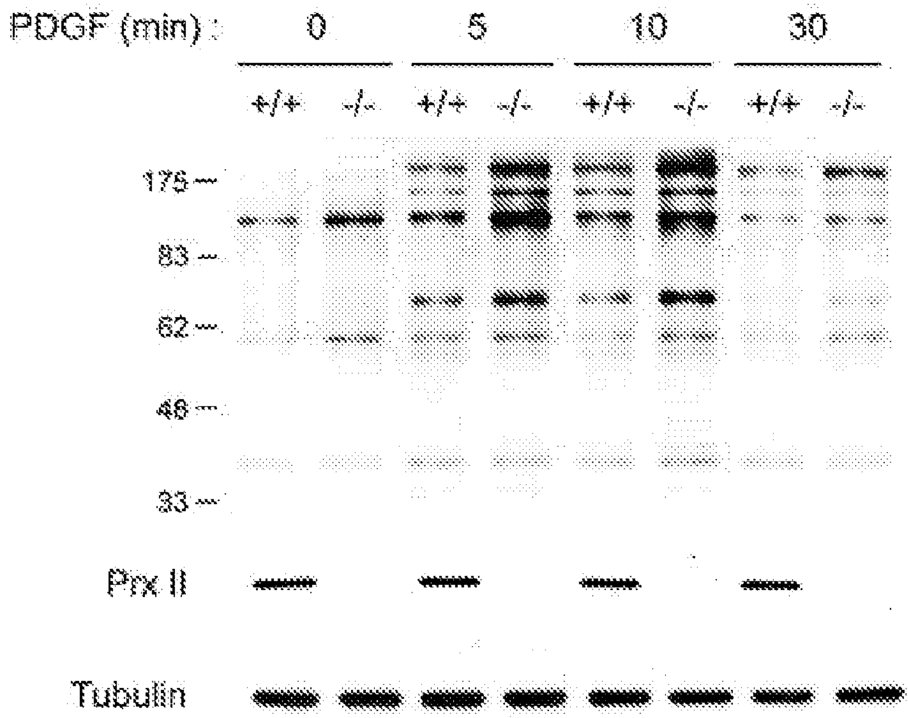
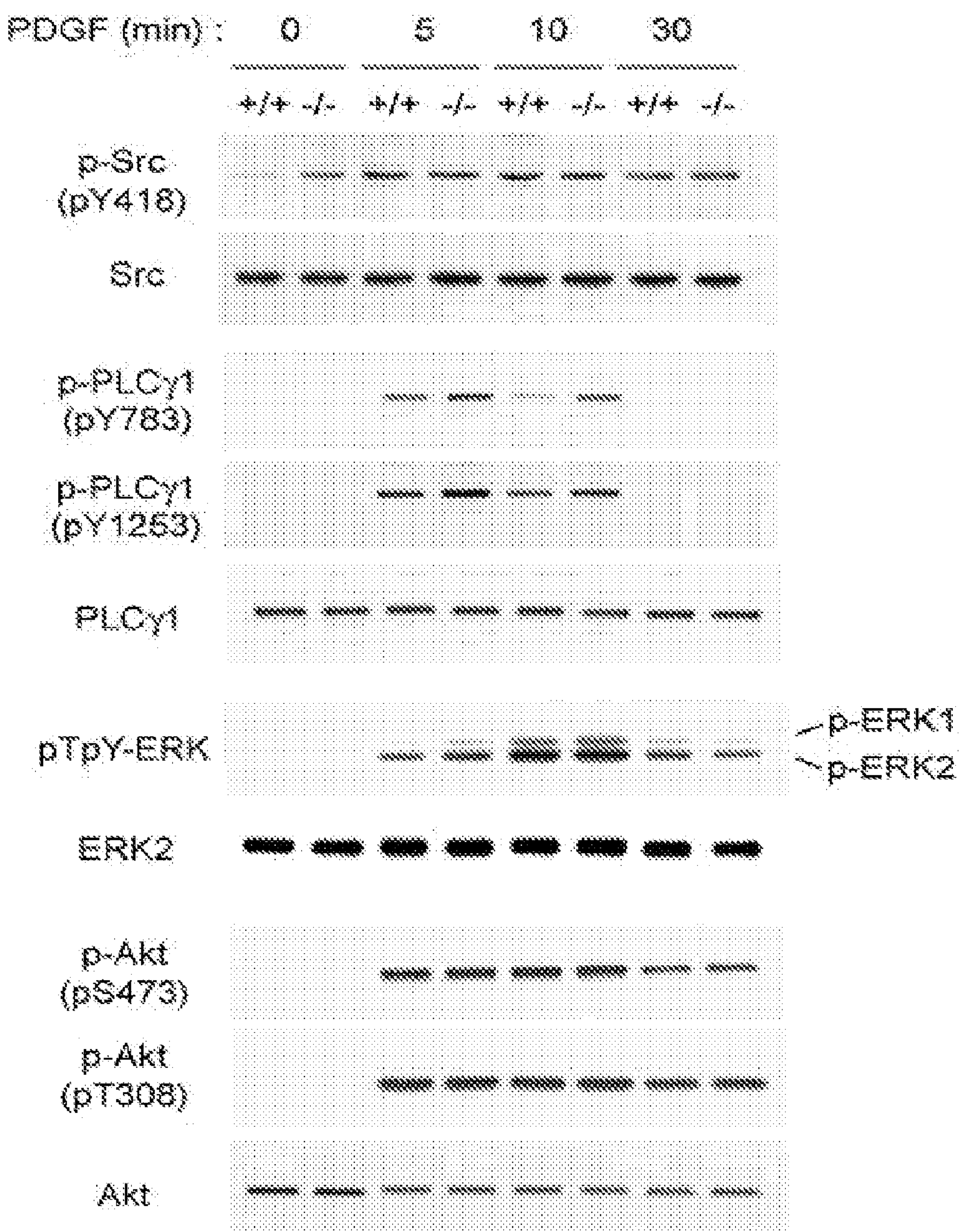


FIG. 3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30
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(pY418)
Src
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(pY783)
p-PLCγ1
(pY1253)
PLCγ1
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ERK2
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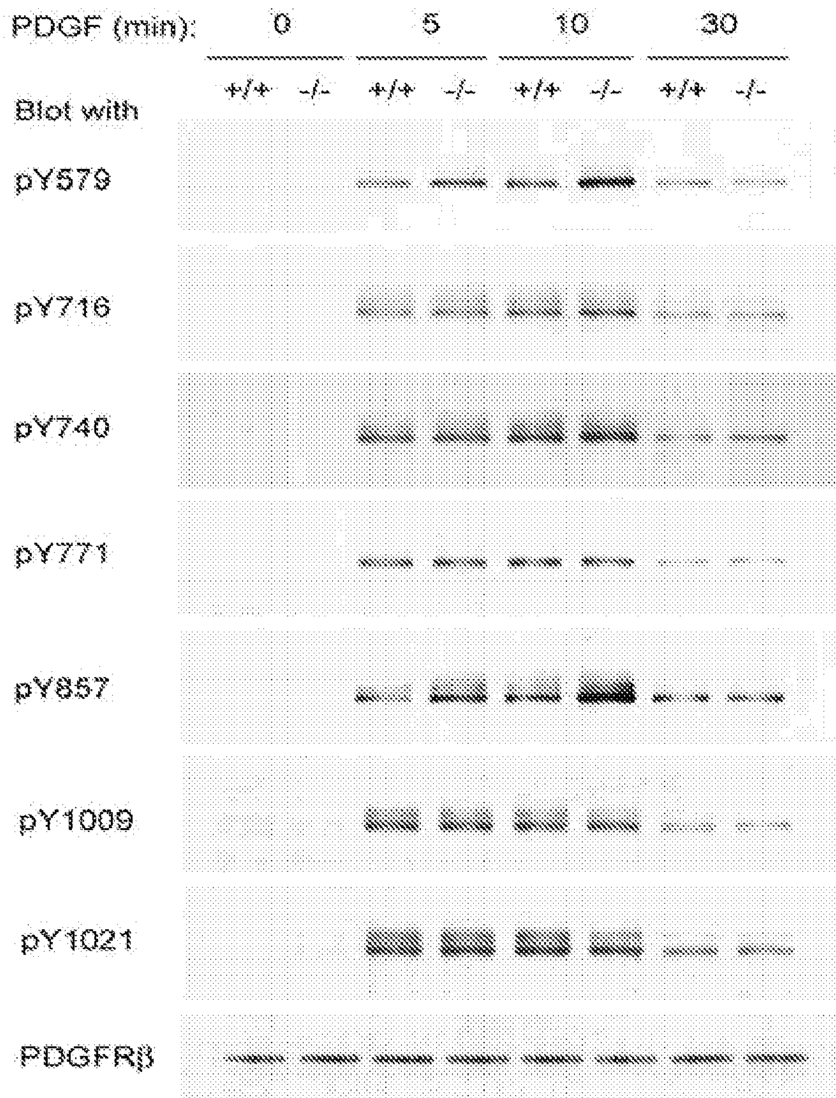
(pS473)
p-Akt
(pT308)
Akt

FIG. 4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10
30
+/+
-/-
+/+
-/-
+/+
-/-
+/+
-/-
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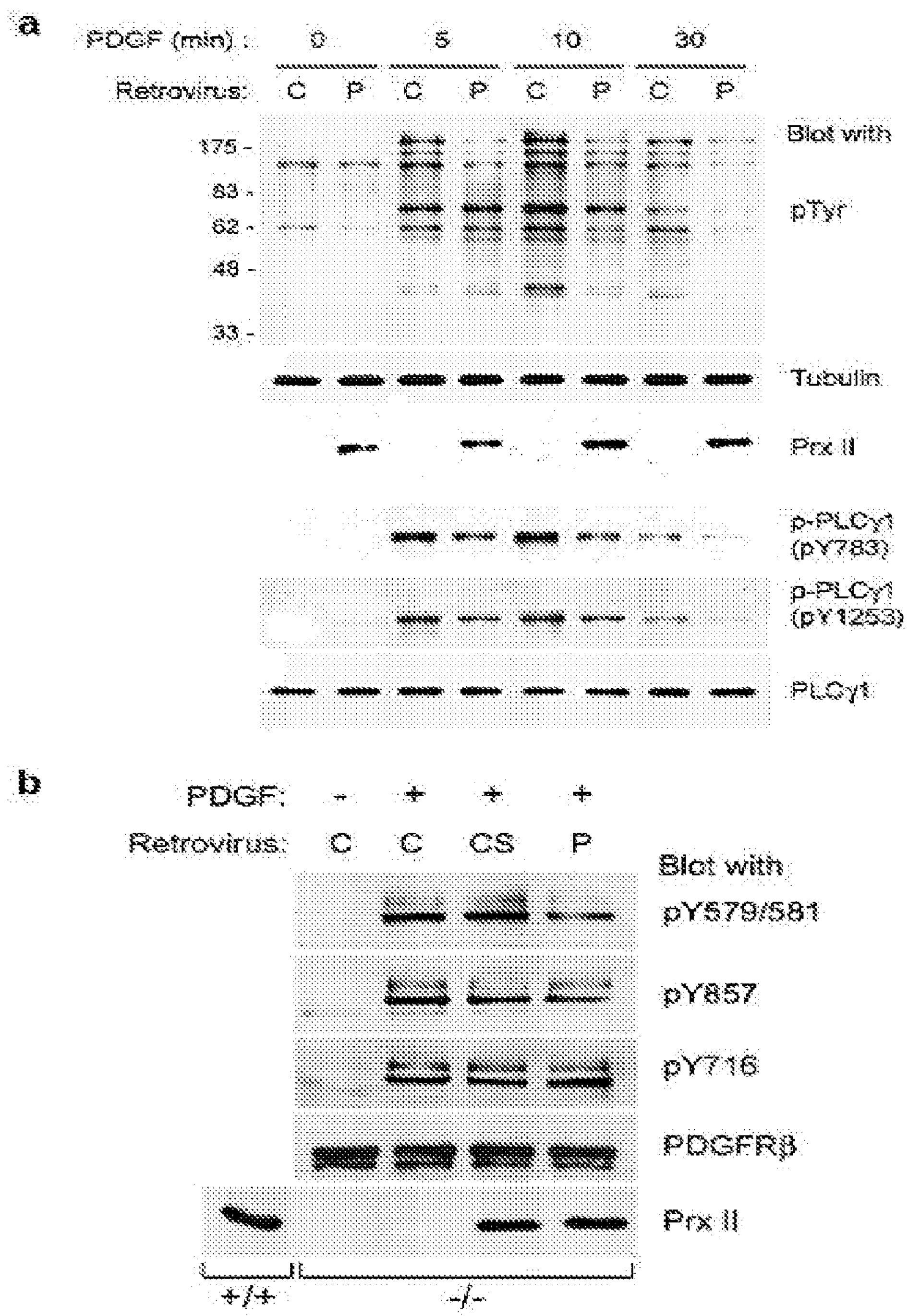
pY740
pY771
pY857
pY1009
pY1021
PDGFRβ

FIG. 5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5
10
30
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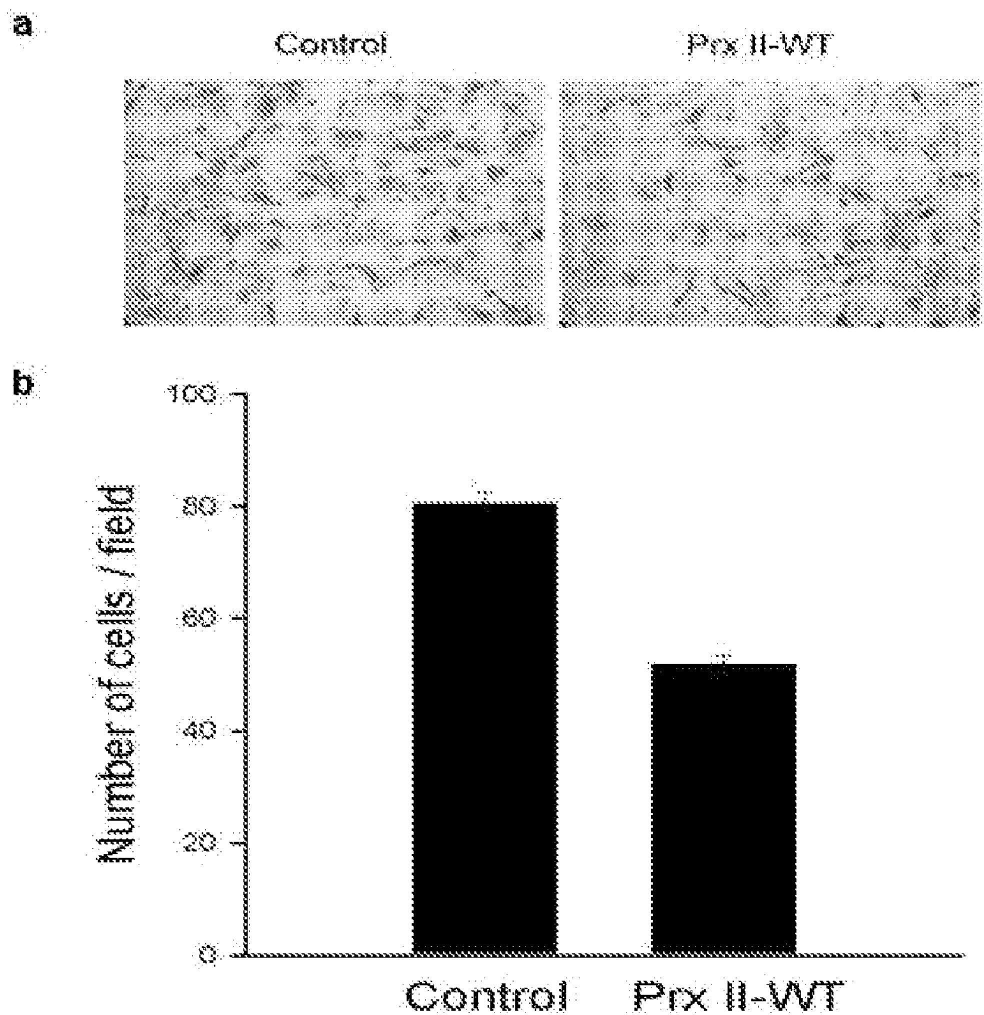
C
P
C
P
C
P
C
P
175 -
83 -
62 -
48 -
33 -
Blot with
pTyr
Tubulin
Prx II
p-PLCγ1
(pY783)
p-PLCγ1
(pY1253)
PLCγ1
b
PDGF:
-
+
+
+
Retrovirus:
C
C
CS
P
Blot with
pY579/581
pY857
pY716
PDGFRβ
Prx II
+/+
-/-

FIG. 6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Prx II-WT
b
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60
40
20
0
Control
Prx II-WT

FIG. 7

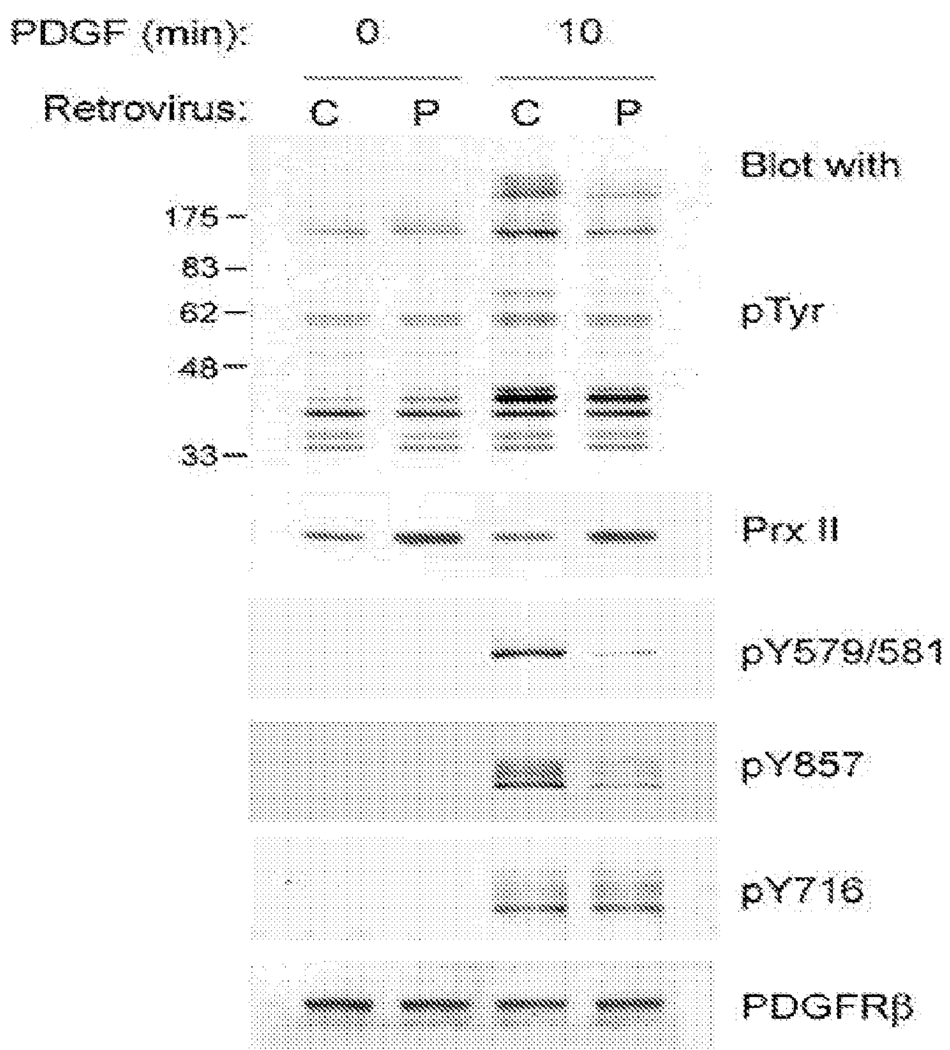
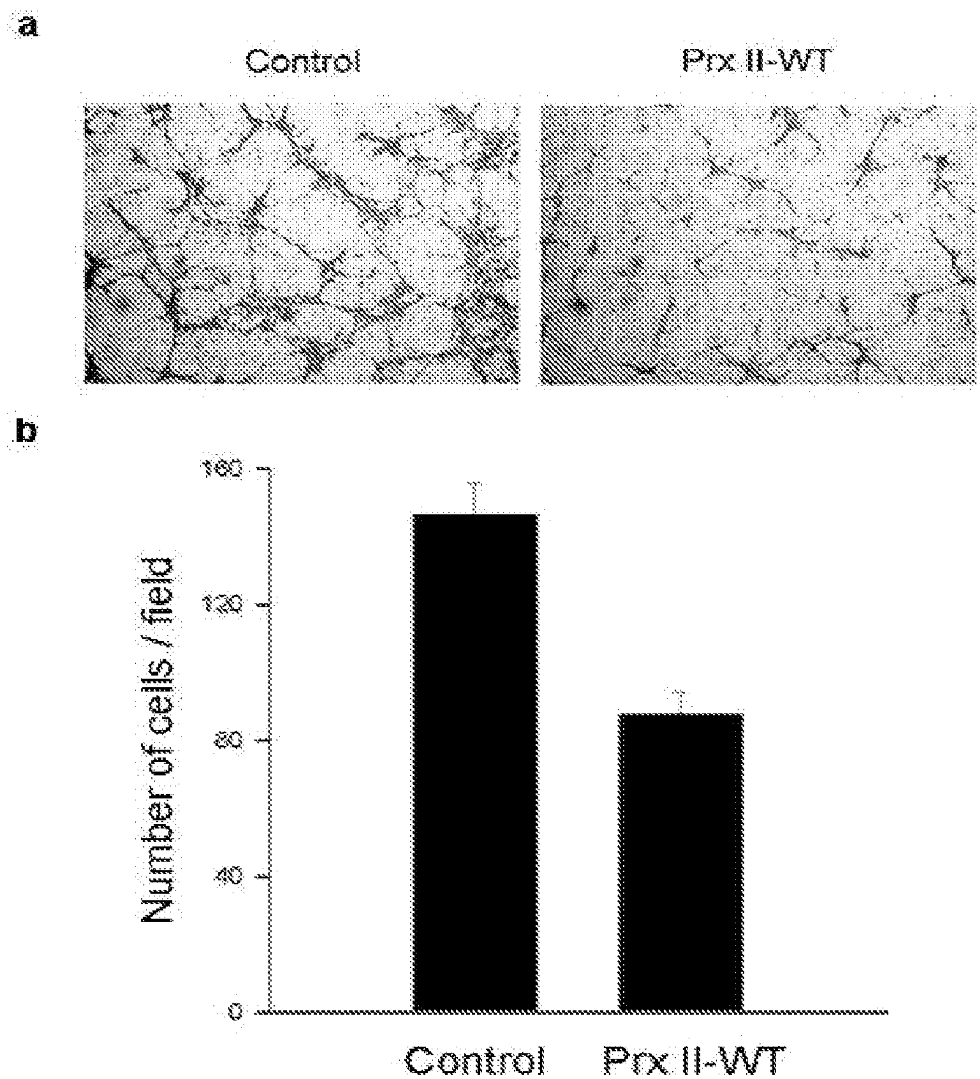

PDGF (min): 0 10
Retrovirus: C P C P
Blot with
175
83
62
48
33
pTyr
Prx II
pY579/581
pY857
pY716
PDGFRβ

FIG. 8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Prx II-WT
b
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120
80
40
0
Number of cells / field
Control
Prx II-WT

FIG. 9

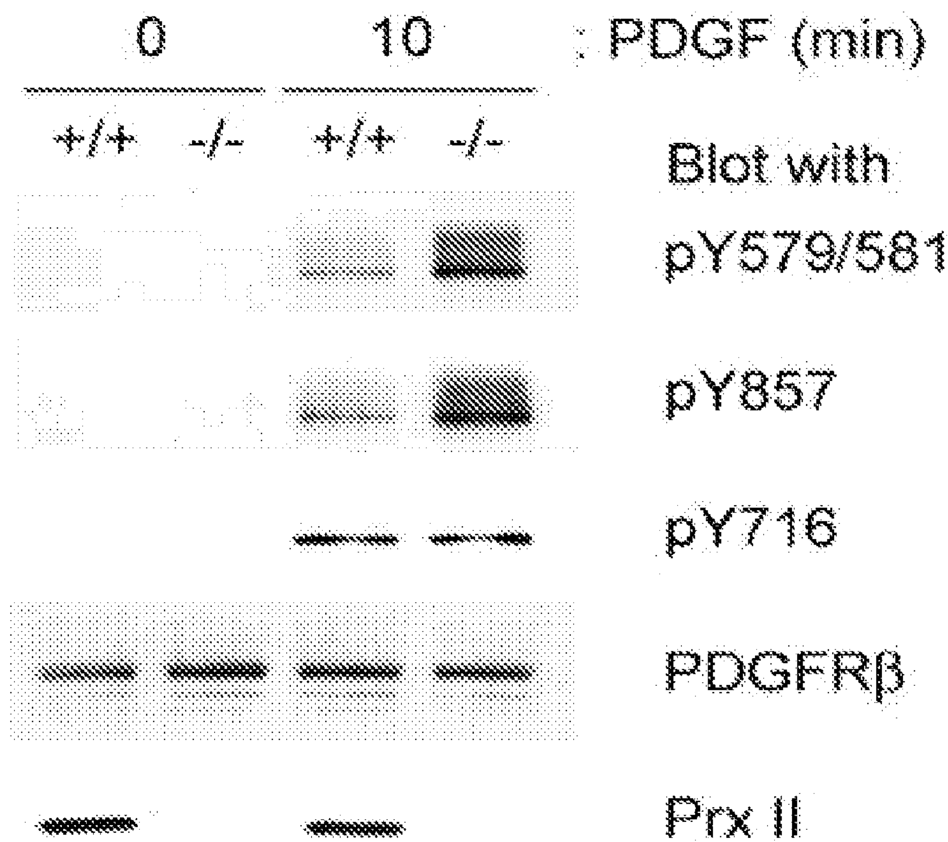


FIG. 10

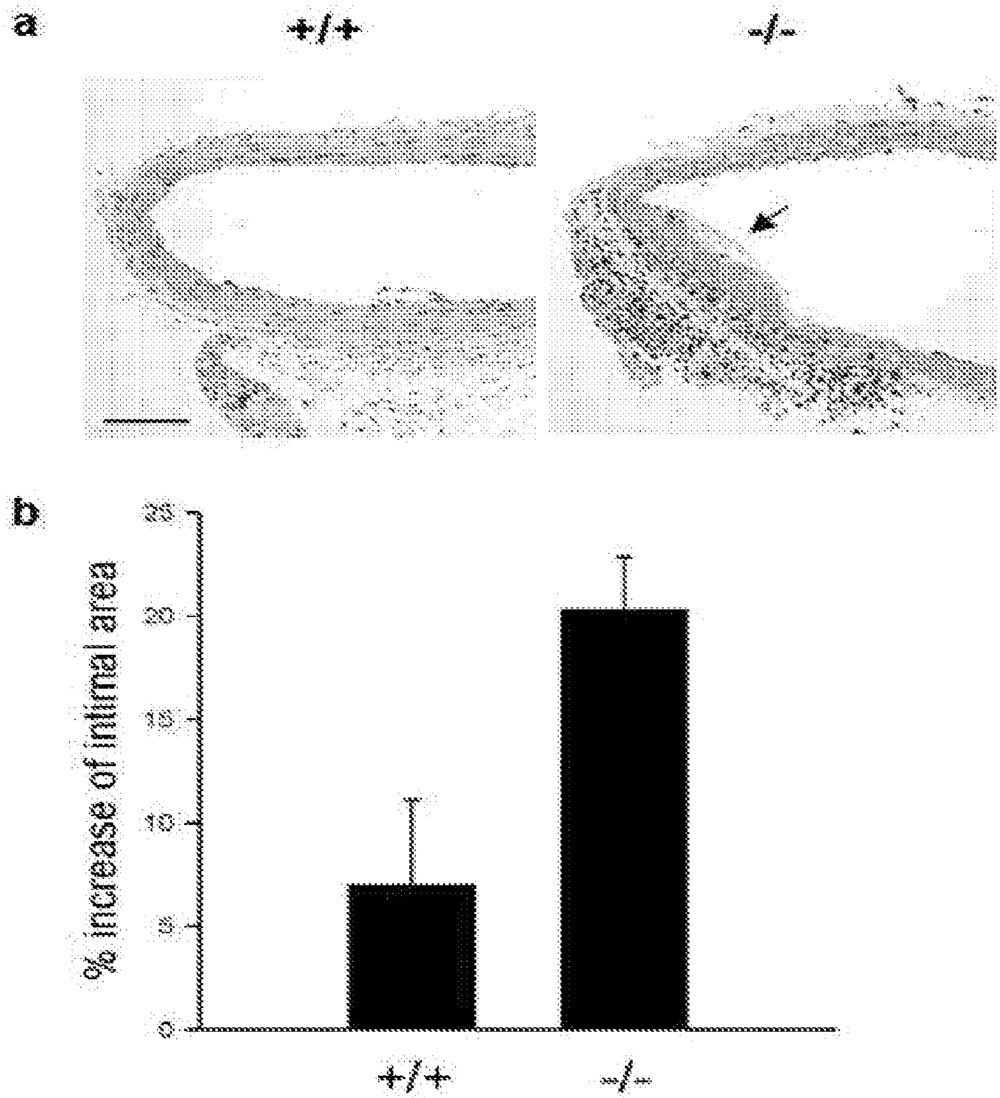
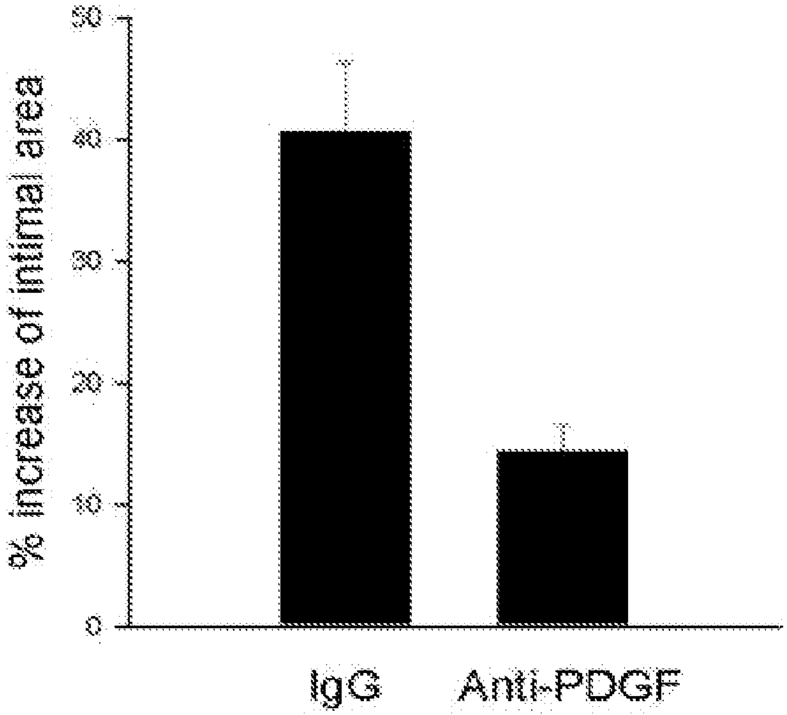


FIG. 11



PHARMACEUTICAL COMPOSITION FOR PREVENTING AND TREATING RESTENOSIS COMPRISING PEROXIREDOXIN 2 PROTEIN AS AN ACTIVE INGREDIENT AND COMPOSITION FOR SCREENING THERAPEUTICS OF RESTENOSIS COMPRISING PEROXIREDOXIN 2 GENE OR ITS PROTEIN PRODUCT, AND METHOD FOR SCREENING THERAPEUTICS OF RESTENOSIS USING SAID COMPOSITION

TECHNICAL FIELD

[0001] The present invention relates to a pharmaceutical composition for the prophylaxis and treatment of restenosis (renarrowing), a composition for screening therapeutics for restenosis, and a method for screening therapeutics for restenosis using the same.

BACKGROUND ART

[0002] Peroxiredoxin (Prx) is a scavenger of hydrogen peroxide and alkyl hydroperoxides in living organisms (Chae, H. Z. et al., Proc. Nat. Acad. Sci. 91: 7017-7021, 1994). There are six distinct mammalian Prx isozymes, types I to VI, that are detected in a wide range of tissues (Rhee, S G et al., IUBMB Life, 52:35-41, 2001). These have been found to have potent antioxidant activities in vivo. All of the known mammalian Prxs, except for type VI, utilize thioredoxin as an electron donor, and thus were formerly known as thioredoxin peroxidases.

[0003] In addition to their antioxidant activity, Prxs have been implicated in various cellular functions including cell proliferation and differentiation, enhancement of natural killer cell activity, protection of radical-sensitive proteins, heme metabolism, and intracellular signaling (Nemoto Y, et al., Gene, 91:261-265, 1990; Prosperi M T, et al., Genomics, 19:236~241,1994; Tsuji K, et al., Biochem J. 307:377~381, 1995; Shau H, et al., Immunogenetics, 40:129~134,1994; Watabe S, et al., Biochem Biophys Res Commun. 213:1010~1016, 1995; Iwahara S, et al., Biochemistry, 34:13398~13406, 1995; Wen S T, et al., Genes Dev. 11:2456~2467, 1997). The biochemical characteristics revealed from cultured animal cell studies show that Prx may be one of those that play important roles in maintaining cellular redox potential.

[0004] Of these antioxidant proteins, 2-cys peroxiredoxin type II (Prx II) is a cellular peroxidase that eliminates endogenous H_2O_2 produced in response to growth factors including platelet-derived growth factor (PDGF) and epidermal growth factor (EGF).

[0005] It is known that Prx II is located in abundance in the cytoplasm of cells, binds to integral membrane proteins or cell membranes via its C-terminal region, and has a high affinity for H_2O_2 (Km for H_2O_2<10 μm).

[0006] Also, Prx II is found to be highly expressed in erythrocytes and to play a protective role against reactive oxygen species (ROS)-mediated damage in these cells, and is induced at the early stages of erythroid differentiation prior to hemoglobin accumulation (Rabilloud T, et al., Biochem J. 312:699-705, 1995).

[0007] Platelet-derived growth factor (PDGF) is a potent mitogenic and migratory factor that regulates the tyrosine phosphorylation of a variety of signaling proteins through the generation of endogenous H_2O_2. Particularly, PDGF stimulates the proliferation and migration of smooth muscle cells during vascular remodeling. In this regard, Prx II serves as an important regulator for the PDGF-mediated generation of H_2O_2, but its precise function in signal transduction has yet to be proven.

[0008] Based on the observation that Prx II−/− mice have hemolytic anemia, Lee T H et al., suggested that Prx II plays a major role in protecting RBCs from oxidative stress in mice (Lee T H et al., Blood, 101(12):pp 5033-5038, 2003). U.S. Pat. Publication No. 2002/0168353 discloses a composition for the treatment of HIV infection, comprising purified type I Peroxiredoxin and type II Peroxiredoxin.

[0009] Restenosis, or renarrowing, is said to recur in as many as 50% of stenosis cases or more, as determined by coronary angiography after coronary angioplasty. Being a major obstacle to the successful treatment of cardiovascular diseases, restenosis occurs in about 30% of patients who receive coronary angioplasty (balloon dilatation and stent insertion).

[0010] Restenosis, although the precise mechanism thereof. must be further revealed, is known to be attributed to the fact that growth factors and cytokines are locally secreted due to vascular endothelial cell injury during angioplasty or balloon dilatation and induce smooth muscle cell proliferation and migration through autocrine and paracrine mechanisms, leading to the narrowing of artery lumens. Hence, smooth muscle cell proliferation has recently been recognized as a clinical problem that is important in the effectiveness of coronary angioplasty (Bauters C, Isner J M, Prog Cardiovasc Dis. 40(2):107-116, 1997, U.S. Pat. Nos. 6,780,406 and 6,740,678).

[0011] Extensive studies have been conducted to reduce or prevent restenosis. Particularly, many attempts have been made to regulate smooth muscle cell proliferation, using angiotensin converting enzyme (ACE) inhibitors, antisense RNAs for cell cycle regulatory proteins, and thymidine kinase genes (Rakugi et al, J. Clin. Invest., 93:339-346, 1994; Simons et al., Nature, 359:67-70, 1992; U.S. Pat. No. 6,780,406).

[0012] Leading to the present invention, the intensive and thorough study on intracellular functions of Prx II, conducted by the present inventors, resulted in the finding that Prx II is involved in vascular muscle cell migration, thereby being used in preventing or reducing vascular restenosis.

DISCLOSURE

Technical Solution

[0013] Accordingly, the present invention has been made keeping in mind the above problems occurring in the prior art, and an object of the present invention is to provide a pharmaceutical composition for the prophylaxis and treatment of vascular restenosis, comprising Prx II as an active ingredient.

[0014] Another object of the present invention is to provide a composition for screening therapeutics for restenosis.

[0015] A further object of the present invention is to provide a method for screening therapeutics for restenosis using the composition.

In accordance with an embodiment, the present invention pertains to a composition for the prophylaxis and treatment of vascular restenosis (renarrowing), comprising Prx II as an active ingredient.

[0016] Particularly, the composition comprising Prx II as an active ingredient in accordance with the present invention may be used for the prophylaxis and treatment of restenosis in the carotid artery, the coronary artery, the peripheral artery, and the renal artery.

[0017] In addition, the composition comprising Prx II as an active ingredient in accordance with the present invention is useful for preventing and treating restenosis attributable to hardening chronically occurring in grafted vessels or angioplasted segments or to short-term proliferation after angioplasty.

[0018] In addition to Prx II, the composition of the present invention may further contain other ingredients having inhibitory activity against restenosis.

[0019] Prx II has inhibitory activity against various cellular events including receptor tyrosine phosphorylation through growth factor signaling, vascular smooth muscle cell proliferation and migration, and neointimal thickening. This protein can be expressed by the base sequence of a prx II gene, listed in SEQ. ID. NO. 1, that encodes the amino acid sequence listed in SEQ. ID. NO. 2.

[0020] Prx II (gene loci Prdx2) is a cellular peroxidase that eliminates endogenous H_2O_2 produced in response to growth factors such as PDGF and EGF. The enzyme suppresses the amplification of intracellular signal transduction by inhibiting site-specific phosphorylation at PDGFRβ-PLCγ1. Through this mechanism, Prx II leads to the suppression of the proliferation, migration and neointimal thickening of vascular smooth muscle cells.

[0021] Upon stimulation with PDGF, the intracellular levels of H_2O_2 and phosphorylated proteins in Prx II-deficient embryonic fibrocytes increase. In Prx II-deficient cells stimulated with PDGF, PLCγ1 (phospholipase gamma 1) is phosphorylated particularly on tyrosine-783 and tyrosine-1253. Also, a phosphorylation increase is found only on tyrosine 579/581 and 857 out of the phosphorylation residues of PDGFRβ. However, the re-expression of wild-type Prx II within the cells returns these cellular events back to the suppression of tyrosine inhibition.

[0022] Prx II was also found to suppress the tyrosine phosphorylation of PDGFRβ in a site-specific manner, as well as cell migration in both human primary smooth muscle cells and mouse embryonic fibroblasts.

After being injured, the carotid artery of Prx II-deficient mice (Prx II−/− mice) undergoes more severe neointimal thickening during restenosis than does that of wild-type mice.

[0023] Taking advantage of the fact that during vascular remodeling, the loss of Prx II functions results in increasing the migration of smooth muscle cells, a pharmaceutical composition comprising Prx II, which suppresses the migration and neointimal thickening of smooth muscle cells, can be used as a medication for the diagnosis, prevention and treatment of cardiovascular diseases caused by smooth muscle cell migration, such as restenosis (Ferns G A, et al., Science. 1991 Sep. 6; 253(5024):1129-32; Heldin C H, Westermark B. Physiol Rev. 1999 October; 79(4):1283-316).

[0024] In the present invention, it is suggested that Prx II is an H_2O_2-based signal regulator that plays a special role in PDGFR signal transduction and can be a new target protein in the treatment of cardiovascular diseases.

[0025] In addition to the active ingredient Prx II, the composition of the present invention may contain pharmaceutically acceptable and physiologically suitable additives. Vehicles, disintegrants, sweeteners, binders, coatings, dilatants, lubricants, talc, and/or flavors may be used in the composition.

[0026] The composition of the present invention comprising Prx II as an active ingredient may be formulated in combination with at least one pharmaceutically acceptable carrier.

[0027] For liquid formulations, physiological saline, sterile saline, Ringer's solutions, buffered saline, albumin injections, dextrose solutions, maltodextrose solutions, glycerol, ethanol and combinations thereof may be used as vehicles. If necessary, other conventional additives such as antioxidants, buffers, bacteriostatics, and the like, may be added. Also, together with diluents, dispersants, surfactants, binders, lubricants and the like, the composition of the present invention may be formulated into pills, capsules, granules, and tablets as well as injection forms, such as solutions, suspensions, emulsions, etc. Further, the composition of the present invention may be formulated according to the methods described in Remington's Pharmaceutical Science, Mack Publishing Company, Easton Pa.

[0028] In accordance with the present invention, the composition comprising Prx II as an active ingredient may be administered in the form of granules, powders, coated tablets, capsules, suppositories, syrups, juices, suspensions, emulsions, injectable solutions, or sustained release agents.

[0029] As for the administration of the composition of the present invention comprising Prx II as an active ingredient, it may be achieved in a conventional manner through one of intravenous, intraarterial, intraabdominal, intramuscular, intrathoracic, transdermal, intranasal, inhalation, local, rectal, oral, intraocular, or subcutaneous routes, or the like.

[0030] Factors determining the dose of the composition comprising Prx II as an active ingredient include the type and severity of disease, the content and kind of the active and other ingredients, the dosage form of the composition, the age, body weight, physical condition, sex and diet of patients, the administration time interval, administration route, release rate of the composition, treatment time periods, co-administered drugs, etc. For adults, the composition may be administered once or many times a day in a total daily dose from 0.1 to 10 mg of Prx II per kg of body weight.

[0031] In accordance with another embodiment, the present invention pertains to a method for preventing and treating cardiovascular diseases caused by smooth muscle cell migration, such as vascular restenosis, by administering Prx II.

[0032] The present invention is intended to suppress the migration or neointimal thickening of smooth muscle cells

after vessel injury in patients with cardiovascular diseases by administering Prx II thereto, thereby preventing vascular restenosis.

[0033] In the method for the prophylaxis and treatment of cardiovascular diseases caused by smooth muscle cell migration, such as restenosis, a Prx II protein may be administered via an intravenous, an intraarterial, an intraabdominal, an intramuscular, an intrathoracic, a transdermal, an intranasal, an inhalation, a local, a rectal, an oral, an intraocular, or a subcutaneous route.

[0034] In the method for the prophylaxis and treatment of cardiovascular diseases caused by smooth muscle cell migration, such as restenosis, Prx II is administered at a total daily dose from 0.01 mg to 100 mg per kg of body weight for adults one or many times a day, depending on various factors, including the type and severity of disease, the content and kind of the active and other ingredients, the dosage form of the composition, the age, body weight, physical condition, sex and diet of patients, the administration time interval, administration route, release rate of the composition, treatment time periods, co-administered drugs, etc.

[0035] In accordance with a further embodiment, the present invention pertains to the use of Prx II in the preparation of medical products for the prophylaxis and treatment of cardiovascular diseases caused by smooth muscle cell migration, such as restenosis.

[0036] In accordance with still a further embodiment, the present invention pertains to a composition for screening therapeutics for restenosis, comprising a mammalian Prx II gene.

[0037] The Prx II gene useful in the screening composition of the present invention may be selected from among the base sequence of SEQ. ID. NO. 1, base sequences of polymorphs of SEQ. ID. NO. 1, fragments of the base sequences, and combinations thereof.

[0038] In accordance with still another embodiment, the present invention pertains to a composition for screening therapeutics for restenosis, comprising a mammalian Prx II protein.

[0039] The Prx II protein useful in the screening composition of the present invention may be selected from among the amino acid sequence of SEQ. ID. NO. 2, proteins expressed from the base sequence of SEQ. ID. NO. 1, proteins expressed from the base sequences of polymorphs of SEQ. ID. NO. 1, Prx II polypeptide fragments having the same physiological activity as Prx II, and combinations thereof.

[0040] The screening composition of the present invention shows a relieving effect on restenosis.

[0041] In accordance with yet another embodiment, the present invention pertains to a method for screening therapeutics for restenosis using as a target material the screening composition comprising a Prx II gene.

[0042] In this method, the composition for screening therapeutics for restenosis comprising a Prx II gene is brought into contact with a test material to determine whether the test material enhances or suppresses the expression of the gene contained in the composition.

In accordance with yet a further embodiment, the present invention pertains to a method for screening therapeutics for restenosis using as a target material the screening composition comprising a Prx II protein.

[0043] In this method, the composition for screening therapeutics for restenosis comprising a Prx II protein is brought into contact with a test material to determine whether the test material enhances or suppresses the expression of the gene contained in the composition.

To screen therapeutics for restenosis with the composition comprising a Prx II gene, conventional methods useful for the analysis of reaction between DNA-DNA, DNA-RNA, DNA-protein, or DNA-compound may be used.

[0044] For example, available is a hybridization test for identifying the combination between the gene and a test material in vitro, a method for measuring the expression rate of the gene through Northern analysis after reaction between mammalian cells and a test material, quantitative PCR, and quantitative in-time PCR, or a reporter gene assay in which the gene coupled with a reporter gene is introduced into cells to react with a test material, followed by measuring the expression rate of the reporter protein.

[0045] In this regard, the composition of the present invention may contain distilled water or buffer for stabilizing the structure of nucleic acids, as well as the Prx II gene.

[0046] To screen therapeutics for restenosis with the composition comprising a Prx II protein, conventional methods useful for the analysis of reaction between proteins and proteins, or between proteins and compounds, may be used.

[0047] Available are, for example, screening methods utilizing the measurement of the activity of a test compound after reaction with the Prx II gene or protein, a yeast two-hybrid assay, a search for a phage-displayed peptide clone binding to a Prx II protein, HTS (high throughput screening) using a natural or chemical library, drug hit HTS, cell-based screening, or DNA array.

[0048] In this regard, the composition of the present invention may contain a buffer or reaction solution for safely maintaining the structure or activity of the protein in addition to the protein expressed from the Prx II gene. For in vivo assays, the composition of the present invention may further comprise a cell capable of expressing the protein or a cell containing a plasmid capable of expressing the protein in the presence of a promoter regulating the description of the gene.

[0049] In the screening method of the present invention, the test material may be a material that is assumed to have the possibility of being used as a therapeutic for restenosis according to conventional assays, or randomly selected nucleic acid sequences, proteins, extracts or natural materials.

[0050] If a test material is found to enhance the expression of the gene or the activity of the protein as measured by the screening method of the present invention, it can be a candidate therapeutic for restenosis. On the other hand, if a test material is found to suppress the expression of the gene or the activity of the protein as measured by the screening method of the present invention, its inhibitors can be also candidates for therapeutics for restenosis.

[0051] As such, the candidates for therapeutics for restenosis in accordance with the present invention may be leading compounds for the development of the therapeutics, which optimally modify the structure of the Prx II gene or protein so as to enhance the expression of the Prx II gene or the activity of the Prx protein.

[0052] Partially or fully enhancing the expression of mammalian Prx II genes or the activity of mammalian Prx II proteins, the materials thus obtained can be used to treat restenosis, coronary sclerosis, or other cardiovascular diseases caused due to the decreased expression of the Prx II gene or the decreased activity of the Prx II protein.

DESCRIPTION OF DRAWINGS

[0053] FIG. **1** shows intracellular H_2O_2 levels in normal MEF and Prx II(−/−) MEF in response to PDGF stimulation.

[0054] FIG. **2** shows levels of phosphorylation on total proteins in normal MEF and Prx II(−/−) MEF in response to PDGF stimulation.

[0055] FIG. **3** shows levels of phosphorylation on individual signaling proteins in normal MEF and Prx II(−/−) MEF in response to PDGF stimulation.

[0056] FIG. **4** shows levels of phosphorylation on various tyrosine residues of PDGFRβ in normal MEF and Prx II(−/−) MEF in response to PDGF stimulation.

[0057] FIG. **5** shows levels of phosphorylation on total proteins and PLCγ1 when a human Prx II wild-type protein is expressed in mouse Prx II(−/−) MEF (a) and levels of phosphorylation on PDGFRβ in response to PDGF when human Prx II wild-type and inactivated mutant proteins were expressed in mouse Prx II(−/−) MEF (b).

[0058] FIG. **6** shows cell migration towards PDGF (a) and the number of cells that migrated (b) when a human Prx II wild-type protein is expressed in mouse Prx II(−/−) MEF.

[0059] FIG. **7** shows levels of phosphorylation on total proteins and tyrosine residues of PDGFRβ in response to PDGF upon the overexpression of a Prx II wild-type protein in human VSMCs in which Prx II wild-type protein is expressed.

[0060] FIG. **8** shows cell migration towards PDGF (a) and the number of cells that migrated (b) when a Prx II protein is overexpressed in human VSMCs.

[0061] FIG. **9** shows the tyrosine phosphorylation of PDGFRβ when VSMCs isolated from Prx II(−/−) mice are treated with PDGF.

[0062] FIG. **10** shows neointimal thickening after vessel injury in normal and Prx II(−/−) mice.

[0063] FIG. **11** shows neoinitmal thickening after vessel injury in Prx II(−/−) mice injected with control IgG and an anti-PDGF antibody.

MODE FOR INVENTION

[0064] A better understanding of the present invention may be obtained through the following examples which are set forth to illustrate, but are not to be construed as the limit of the present invention.

Example 1

Effect of Prx II on Tyrosine Phosphorylation of Endogenous Proteins

[0065] 1-1) Observation of H_2O_2 Generation and Tyrosine Phosphorylation in Response to PDGF with Respective to Prx II Expression

In this example, changes in endogenous H_2O_2 generation in response to PDGF were observed with respect to Prx II expression.

[0066] Wild-type Prx II(+/+) MEF (mouse embryonic fibroblasts) and Prx II(−/−) MEF were prepared according to a typical process well known in the art after Prx II(+/−) mice were mated with each other and embryos were excised from the mice on the 10th day of pregnancy (Kang, S. W. et al., J Biol Chem, 279, 2535-43 (2004)). PDGF-BB (25 ng/ml, Upstate) was used for PDGF stimulation.

[0067] The intracellular level of H_2O_2 was measured using 2,7-dichlorofluorescin diacetate (H2DCFDA, Molecular Probes Inc.).

[0068] When applied to cells, this compound crosses the plasma membrane and is transported into cells wherein it is hydrolyzed into impenetrable 2',7'-dichlorofluorescin (H2DCF) by endogenous esterases. This hydrolysate is oxidized by endogenous H_2O_2 to form 2',7'-dichlorofluorescein (DCF) that has an excitation peak at 488 nm and an emission peak at 515 nm, enabling fluorescence detection.

[0069] Prx II(+/+) MEF and Prx II(−/−) MEFs were cultured in DMEM supplemented with 10% FBS (fetal bovine serum) in the presence of an antibiotic. Prior to PDGF stimulation, these cells were synchronized by serum starvation for 24 hours in DMEM supplemented with 0.5% FBS. PDGF stimulation was performed by treating the cells with PDGF at a density of 25 ng/ml for 5, 10, or 30 min. After completion of PDGF stimulation, the cells were washed with HBSS (HEPES-buffered saline solution) and allowed to react for an additional 5 min in 5 mM DCFH-DA. Fluorescence was measured using a confocal laser microscope (Kang, S. W. et al. J Biol Chem 273, 6297-302 (1998)).

[0070] In FIG. **1***a*, there are photographs of 2',7'-DCF fluorescence taken 10 min after the PDGF-BB stimulation, showing that Prx II(−/−) MEFs are higher in endogenous H_2O_2 level than Prx II(+/+) MEFs both before and after PDGF stimulation.

[0071] In FIG. **1***b*, relative intensities of DCF are plotted against time, showing that the generation of H_2O_2 in the Prx II-deficient mouse cells was about twice as large as that in wild-type cells in response to PDGF and that the generation of H_2O_2 was temporally increased and returned to the base line within 30 min.

[0072] 1-2) Observation of Tyrosine Phosphorylation of Endogenous Proteins

Following the treatment of Prx II(+/+) MEF and Prx II(−/−) MEF with PDGF as in Example 1-1, the phosphorylation of intracellular proteins over time was observed.

[0073] Proteins extracted from each cell were immunoblotted with an anti-phosphorylation antibody (4G10, Upstate) so as to analyze them for tyrosine phosphorylation. An anti-Prx II antibody (Labfrontier Co., Korea), and an

anti-tubulin antibody (Sigma-Aldrich Co.) was used for immunoblotting and the results are given in FIG. 2.

[0074] Coincident with Example 1-1 with respect to H_2O_2 generation over time, the PDGF-induced tyrosine phosphorylation on proteins in the Prx II(−/−) MEFs was observed to dramatically increase compared to that in the wild-type MEFs (FIG. 2).

[0075] 1-3) Observation of the phosphorylation of phospholipase C gamma 1 Using phospho-specific antibodies, Prx II expression was analyzed for the activation of downstream signaling molecules.

[0076] After treatment with PDGF, Prx II(+/+) MEFs and Prx II(−/−) MEFs were immunoblotted to determine phosphorylation on Src, PLCγ1, ERK2, and Akt proteins with time. In this regard, phospho-Src antibodies (Biosource), phospho-Akt antibodies (Cell Signaling Technology), and phospho-ERK antibodies (Cell Signaling Technology) were used and c-Src, Akt, PDGFRβ (M-20), and ERK2 were purchased from Santa Cruz Biotechnology. Anti-phospho-PLCγ1 (pY783 and pY1253) antibodies were prepared according to well-known methods (Sekiya F, et al., J Biol. Chem. 2004 279(31):32181-90).

[0077] As seen in FIG. 3, the residues Tyr783 and Tyr1253, known to be responsible for the activity of PLCγ1, were phosphorylated at higher rates in Prx II(−/−) MEFs than in the wild-type cells, but there were no changes in c-Src, ERK and Akt activation-dependent phosphorylation.

[0078] 1-4) Regulation of Prx II in Site-Specific Phosphorylation for PDGFR Activation

[0079] 1-4-1) Antibody Preparation

[0080] Phosphor-specific antibodies against peptide regions corresponding to the seven well-known tyrosine residues of PDGFR (providing binding sites for signaling molecules having SH2 domains, such as c-Src, p85 subunit of PI-3K, GAP, Grb2, SHP-2, and PLCg, respectively) were prepared.

[0081] Phospho-peptide antigens (SynPep, USA) used for the production of phospho-specific PDGFR antibodies were as follows: pY579, DGHEpYIYVDPMQ; pY716, SAELpY-SNALPVG; pY740, SDGGpYMDMSKDE; pY751, ESVD-pYVPMLDMK; pY771, ESSNpYMAPYDNY; pY857, RDSNpYISKGSTF; pY1009, SSVLpYTAVQPNE; pY1021, GDNDpYIIPLPDP; (the phosphorylated tyrosine residue in each human PDGFR amino acid sequence was italicized).

[0082] The phospho-peptides were conjugated with keyhole limpet hemocyanin via glutaraldehyde and injected into rabbits. Rabbit anti-serum was purified using sequential affinity chromatography in columns filled with non-phosphorylated and phosphorylated peptide-conjugated Affigel-15 (Bio-Rad) agarose resins.

[0083] 1-4-2) Regulation of Prx II in Site-Specific Phosphorylation for PDGFRβ Activation

[0084] After being treated with PDGF, Prx II(+/+) MEF and Prx II(−/−) MEF cells were analyzed for PDGFRβ phosphorylation over time through an immunoblotting assay. Using anti-phospho −PDGFRβ antibodies, site-specific regulation was observed on the tyrosine residues. As seen in FIG. 4, a significant increase in phosphorylation was observed only on Tyr 579/581 and Tyr 857 in Prx II(−/−) MEFs as compared with wild-type cells.

[0085] When Prx II is deficient, as described above, endogeneous H_2O_2 is not eliminated so that downstream signaling molecules are activated. In this regard, site-specific phosphorylation was observed on the residues Y783 and Y1253 of PLCγ1 and the residues Y579/581 and Y857 of PDGFR. Also, the peroxidase activity of Prx II is essential for the site-specific regulation of PDGFR phosphorylation.

Example 2

Observation of Cell Migration in Prx II-Reexpressed Prx II(−/−) MEF

[0086] An add-back rescue experiment, in which retrovirus was used to re-express human Prx II-wt in Prx II(−/−) MEFs, was performed to examine the influence of the re-expression on protein phosphorylation and migration.

[0087] 2-1) Re-expression of human Prx II-wt in Prx II(−/−) MEFs and observation of protein phosphorylation

[0088] Prx II(−/−) MEF was infected with retrovirus carrying a control gene or a human Prx II wild-type gene in order to examine the phosphorylation of cellular proteins.

[0089] Control retrovirus (C) and human Prx II-encoding retrovirus (P) were obtained from a stably transformed PT67 cell line and a transiently transformed Phoenix-ampho packing cell line (http://www.stanford.edu/group/nolan/index.html).

[0090] In advance of serum starvation, Prx II(−/−) MEFs were infected with human Prx II-encoding retrovirus (P) at 10 M.O.I. (multiplicity of infection) for two days to express Prx II therein.

[0091] Proteins were extracted from the prepared cells and analyzed for total phosphorylation and tyrosine phosphorylation on PLCγ1 and PDGFRβ through immunoblotting assay.

[0092] In response to PDGF, as is apparent from the data of FIG. 5*a*, the Prx II-reexpressed cell was phosphorylated to a lesser extent than was the control cell, not only with respect to total proteins but also with respect to the Y783 and Y1253 of PLCγ1.

[0093] Also, as seen in FIG. 5*b*, the level of the reexpressed Prx II(−/−) MEF was similar to the wild-type MEF (Prx II(+/+)) with respect not only to intracellular Prx II level, but also to tyrosine phosphorylation of PDGFRβ in response to PDGF. Also, Prx II-reexpressed cells were found to have the Y579/581 and Y857 of PDGFR phosphorylated to a lesser extent.

[0094] Therefore, the re-expression of Prx II leads to the suppression of the tyrosine phosphorylation on PLC gamma and PDGFRβ.

[0095] 2-2) Test for Migration of Mouse Embryonic Fibroblast (MEF)

The Prx II(−/−) MEF, in which Prx II was reexpressed in the same manner as above, was examined for cell migration.

[0096] An assay for MEF cell migration was performed in a 24-well Transwell culture chamber (Costar; 8-μm pore

size). The membrane of the Transwell culture chamber was coated with gelatin B (1 μg/μl) and placed on a 24-well plate. In the lower compartment of the culture chamber, PDGF-BB (25 ng/ml) and DMEM, containing 0.1% bovine serum albumin were filled.

[0097] In the upper compartment of the chamber, MEFs (5×10^4) were incubated at 37° C./5% CO_2 for 6 hours. The cells that moved toward the lower part of the filter were stained with 0.6% hematoxylin and 0.5% eosin and counted under an optical microscope. Cell counts were performed in four fields of view selected at random through two independent experiments and were expressed as means ±S.D.

[0098] As understood from data of FIG. **6**, lower counts resulted for the wt Prx II-reexpressed cells than for the control, implying that Prx II suppressed PDGF-induced fibroblast proliferation and migration.

Example 3

Effect of Prx II on Vascular Smooth Muscle Cell Migration

[0099] PDGF is an important factor for smooth muscle cell proliferation and migration during vascular remodeling. The selective regulation of Prx II in the PDGFRβ-PLCγ1 pathway of human and murine vascular smooth muscle cells (VSMCs) was observed in vitro.

[0100] 3-1) Prx II Expression and Cellular Protein Phosphorylation in Human VSMCs

[0101] Human aortic smooth muscle cells were cultured according to the instructions of the manufacturer (Clonetics Co., USA).

[0102] The cells were sub-cultured four times and immunoblotted with an antibody against α-smooth muscle actin (Sigma). Prior to PDGF-BB (25 ng/ml, Upstate) stimulation, the primary cells were subjected to serum starvation (0.5% FBS) for 24 hours.

[0103] Control retrovirus (C) and human Prx II-encoding retrovirus (P) were obtained from a stably transformed PT67 cell line and a transiently transformed Phoenix-ampho packing cell line (http://www.stanford.edu/group/nolan/index.html).

[0104] In advance of serum starvation, VSMCs were infected with human Prx II-encoding retrovirus (P) at 10 M.O.I. (multiplicity of infection) for two days.

[0105] As understood from data of FIG. **7**, neither the control (C) nor the human Prx II-encoding retrovirus-treated group (P) was phosphorylated on tyrosine residues 579/581, 857, and 716 of PDGFRβ before PDGF stimulation. 10 min after PDGF stimulation, phosphorylation on the Y579/581, and Y857 residues of PDGFRβ occurred to a significantly lesser extent in the human Prx II-encoding retrovirus-treated group (P) than in the control (C).

[0106] The overexpression of wild-type Prx II in human aortic VSMCs about three times as high as the endogenous expression of Prx II resulted in a significant decrease in phosphorylation on PLCγ1 and the Y579/581 and Y857 of PDGFR.

[0107] 3-2) Test for Human VSMC Migration

[0108] In the human aortic smooth muscle cells prepared in Example 3-1), Prx II was examined for cell migration.

[0109] An assay for the human aortic smooth muscle cell migration was performed in a 24-well Transwell culture chamber (Costar; 8-μm pore size). The membrane of the Transwell culture chamber was coated with gelatin B (1 μg/μl) and placed on a 24-well plate. In the lower compartment of the culture chamber, PDGF-BB (25 ng/ml) and DMEM, containing 0.1% bovine serum albumin, were charged.

[0110] In the upper compartment of the chamber, VSMCs (5×104) were incubated at 37° C./5% CO_2 for 6 hours. The cells that moved toward the lower part of the filter were stained with 0.6% hematoxylin and 0.5% eosin and counted under an optical microscope. Cell counts were performed in four fields of view selected at random through two independent experiments and were expressed as means ±S.D.

[0111] As can be understood from data of FIG. **8**, lower counts resulted for the wt Prx II-reexpressed cells than for the control, implying that Prx II suppressed PDGF-induced smooth muscle cell migration.

[0112] 3-3) Observation of Phosphorylation in Murine VSMCs

[0113] From wild-type mice and Prx II-deficient mice, both 10 weeks old, aortic smooth muscle cells were prepared according to a method described in the reference (Ohmi, K. et al., Biochem Biophys Res Commun, 238, 154-8 (1997)).

[0114] Proteins were extracted from each cell treated with PDGF and analyzed for the tyrosine phosphorylation of PDGFRβ by immunoblotting.

[0115] When treated with PDGF, as seen in FIG. **9**, VSMCs prepared from the artery of Prx II(−/−) mice showed more intensive site-specific phosphorylation of PDGFRβ than did those prepared from the artery of wild-type mice.

[0116] 3-4) Effect of Prx II on Restenosis of Mouse Carotid Artery after Vessel Injury

[0117] After wild-type and Prx II(−/−) mouse carotid arteries were injured, the effect of Prx II on the neointimal thickening of VSMCs during restenosis was observed in vivo.

[0118] Transluminal wire injuries to mouse left common carotid arteries were performed as specified in the literature (Schober, A. et al., Circulation, 109, 380-5 (2004)).

[0119] 10-week-old male mice were anesthetized, followed by exposing the left external carotid artery and coagulating its branches using electric currents. A 0.016-inch flexible angioplasty guide wire was advanced 1 cm through the transverse arteriotomy of the external carotid artery, and endothelial denudation was achieved by 6 passes along the common carotid artery. After one week, the common carotid artery was excised after transcardiac perfusion-fixation with 3.7% formaldehyde and heparinized saline and then paraffin-embedded.

[0120] 5 consecutive tissue slices (taken at 100 μm intervals and each 3 μm thick) were obtained from the middle region of the common carotid artery. For morphological observation, each slice was stained with hematoxylin and

eosin. Cross-sectional areas of the medial and neointimal layers were analyzed with a computerized analysis system and program (MetaMorpho imaging v5.0, Molecular Devices) with the aid of a digital camera system (CoolSNAP camera system with BX51 microscopy, Olympus).

[0121] Neither the wild-type mice nor the Prx II(−/−) mice underwent the neointimal thickening of the carotid artery that was not injured. When injured with the wire, the carotid artery of the Prx II(−/−) mice had neointimal layers significantly thicker than those of the wild-type mice, as shown in FIG. **10***a*. VSMCs were accumulated on the neointimal layer, as indicated by the arrow in FIG. **10***a*.

[0122] The bar graph of FIG. **10***b* was drawn on the basis of percentage increase of neointimal plaque area relative to medial area (mean ±S.E.M., n=5, P<0.01). As seen in the graph, the % increase of neointimal area of Prx II(−/−) mice increased to about three times that of wild-type mice.

[0123] Therefore, the loss of Prx II function leads to the promotion of VSMC migration during vascular remodeling.

Example 4

Plasma PDGF Neutralization

[0124] In order to prevent the neointimal thickening caused by PDGF-induced signal transduction activation, a test for plasma PDGF neutralization was performed.

[0125] control IgG or an anti-PDGF antibody (Cat. No. AF-220-NA, R&D systems) was injected into Prx II(−/−) three times a day over one week starting one day before injuring the carotid artery. At a dose of 5 μg per 20 g of body weight per injection, the antibody was injected intravenously on −1 and 0 day and then subcutaneously until +4 day. Afterwards, the left external carotid artery was observed for change in neointimal thickness and the results were expressed as percentages of neointimal plaque thickness relative to the medial layer (mean ±S.E.M., n=4, P<0.01).

[0126] As is understood from data of FIG. **11**, anti-PDGF antibody administration significantly reduces the neointimal thickening.

[0127] Prx II suppresses PDGF-induced smooth muscle cell migration while the loss of Prx II functions results in improving smooth muscle cell migration during vascular remodeling. Thus, the pharmaceutical composition of the present invention can be useful for the prophylaxis and treatment of restenosis.

[0128] Also, the composition comprising a Prx II gene or protein in accordance with the present invention can be used for screening materials, that can react therewith, as candidates for developing therapeutics for restenosis.

INDUSTRIAL APPLICABILITY

[0129] As described above, the present invention provides a pharmaceutical composition, comprising Prx II as an active ingredient, for the prophylaxis and treatment of restenosis, based on the research finding that the function of Prx II is highly responsible for the migration of smooth muscle cells during vascular remodeling.

[0130] Further, the screening composition and method according to the present invention is very useful in searching for and developing therapeutics for restenosis.

[0131] Although the preferred embodiments of the present invention have been disclosed for illustrative purposes, those skilled in the art will appreciate that various modifications, additions and substitutions are possible, without departing from the scope and spirit of the invention as disclosed in the accompanying claims.

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1. A media receiver, comprising:

a processor;

a memory in communication with the processor;

a memory card reader in communication with the processor and memory; and

program instructions stored in memory and executable on the processor to update firmware on the media receiver based on a comparison of a firmware version read from a memory card in the memory card reader with a firmware version on the media receiver;

wherein the media receiver includes a receiver selected from the group of:

a digital versatile disk player;

a digital camera;

an MP3 player;

a high definition television (HDTV); and

a HDTV tuner set top box.

2. The media receiver of claim 1, wherein the memory card reader is a seven in one memory card reader.

3. The media receiver of claim 1, wherein the memory card includes a memory card selected from the group of:

a memory stick;

a secure digital card;

a compact flash card;

a smart media card;

an extreme digital picture card; and

a multimedia card.

4. A multimedia device, comprising:

a processor;

a memory in communication with the processor;

a memory card reader in communication with the processor and memory; and

program instructions stored in memory and executable on the processor to:

read a firmware version from a memory card in the memory card reader of the multimedia device; and

select whether to update a firmware version in the memory of the multimedia device based on a comparison of the firmware version on the memory card to the firmware version in the memory of the multimedia device,

wherein the multimedia device includes a device selected from the group of:

a digital versatile disk player;

a digital camera;

an MP3 player;

a high definition television (HDTV); and

a HDTV tuner set top box.

5. The multimedia device of claim 4, wherein the memory card reader is capable of receiving and reading multiple memory card formats.

6. The multimedia device of claim 5, wherein the multiple memory card formats include formats associated with:

a memory stick;

a secure digital card;

a compact flash card;

a smart media card;

an extreme digital picture card; and

a multimedia card.

7. The multimedia device of claim 4, wherein the program instructions can execute to update a firmware version on the multimedia device without using wired connections to a host computer.

8. (canceled)

9. The multimedia device of claim 8, wherein the multimedia device is capable of receiving and reading a firmware update without using a PC software utility.

10. The multimedia device of claim 8, wherein the multimedia device is capable of receiving and reading a firmware update without using a software utility configuration for baud rate, stop bit, and IP address information.

11. A multimedia device, comprising:

a processor;

a memory in communication with the processor; and

means for updating firmware on the multimedia device from a memory card;

wherein the multimedia device includes a device selected from the group of:

a digital versatile disk player;

a digital camera;

an MP3 player;

a high definition television (HDTV); and

a HDTV tuner set top box.

12. The multimedia device of claim 11, wherein the means includes a memory card reader capable of reading multiple memory card formats.

13. The multimedia device of claim 12, wherein the means includes program instructions stored in memory and executable by the processor to read a firmware version stored on a memory card.

14. The multimedia device of claim 13, wherein the means includes program instructions stored in memory and executable by the processor to compare the firmware version read from the memory card in the memory card reader with a firmware version in the memory on the multimedia device.

15. The multimedia device of claim 14, wherein the multimedia device includes input and display means, and wherein the program instructions execute to select whether to update the firmware version in the memory on the multimedia device with the firmware version on the memory card based on user input to a result of version comparison.

16. The multimedia device of claim 15, wherein the input and display means includes a touch screen display on the multimedia device.

17. The multimedia device of claim 15, wherein the display means includes a television screen on a television set connected to the multimedia device and the input means is selected from the group of:

a remote control capable of interacting with the television set; and

an input button on the multimedia device.

18. A method for updating firmware to a multimedia device, comprising:

reading a firmware version from a memory card in a memory card reader of the multimedia device; and

selecting whether to update a firmware version in a memory of the multimedia device based on a comparison of the firmware version on the memory card to the firmware version in the memory of the multimedia device;

wherein the multimedia device includes a device selected from the group of:

a digital versatile disk player;

a digital camera;

an MP3 player;

a high definition television (HDTV); and

a HDTV tuner set top box.

19. The method of claim 18, further including displaying a result from comparing the firmware version on the memory card to the firmware version in the memory of the multimedia device.

20. The method of claim 19, further including selecting whether to update the firm ware version in the memory of the multimedia device based on user input to the multimedia device.

21. The method of claim 18, further including:

downloading a firmware update for the multimedia device to a hard disk of a personal computer via a network connection;

transferring the firmware update from the hard disk in the personal computer to a memory card via a memory card reader in the personal computer;

removing the memory card from the personal computer; and

mounting the memory card in the memory card reader of the multimedia device.

22. The method of claim 21, further including downloading the firmware update via an Internet connection.

23. A method for updating firmware to a multimedia device, comprising:

using a memory card format to perform firmware updates to a multimedia device through a data port; and

performing all update operations local to the multimedia device;

wherein the multimedia device includes a device selected from the group of:

a digital versatile disk player;

a digital camera;

an MP3 player;

a high definition television (HDTV); and

a HDTV tuner set top box.

24. The method of claim 23, the method further including allowing a user to remotely provide input instructions to the multimedia device for selecting whether to perform a firmware update based on a comparison of a firmware version on a memory card to a firmware version resident in a memory of the multimedia device.

25. A computer readable medium having instructions for causing a multimedia device to perform a method, comprising:

reading a firmware version from a memory card in a memory card reader of the multimedia device; and

selecting whether to update a firmware version in a memory of the multimedia device based on a comparison of the firmware version on the memory card to the firmware version in the memory of the multimedia device;

wherein the multimedia device includes a device selected from the group of:

a digital versatile disk player;

a digital camera;

an MP3 player;

a high definition television (HDTV); and

a HDTV tuner set top box.

26. The medium of claim 25, wherein the method further includes displaying a result from comparing the firmware version on the memory card to the firmware version in the memory of the multimedia device.

27. The medium of claim 26, wherein the method further includes initiating an update of the firmware version in the memory of the multimedia device based on user input to the multimedia device.

28. The medium of claim 25, wherein the method further includes remotely selecting whether to update the firmware version in the memory of the multimedia device with the firmware version on the memory card.

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